responsible for the phosphorylation of ADP.[22] He employed a technique in which he fractionated mitochondria after breaking them with glass beads in a vacuum.[23] This produced a red-brown, gelatinous residue or particulate fraction that could oxidize some citric acid cycle intermediates but not accomplish phosphorylation unless the faintly turbid yellow supernatant was re-added. Then phosphorylation increased until reaching a P:O ratio of 0.5. He set about purifying the substance in the supernatant, which he labeled *coupling factor* F_1. At this point Racker followed up on a suggestion first advanced by Henry Lardy and Conrad Elvehjem (1945) that phosphorylation might be the inverse of the breakdown of ATP to ADP, attributed to the enzyme ATPase. He found that F_1 also exhibited ATPase activity, and after showing that both F_1 coupling and ATPase activity decayed at the same rate around 0°, identified them as the same protein (see Penefsky et al., 1960; Pullman et al., 1960; Racker, 1965, Chapter 13). Racker concluded, "F_1 catalyzes the transphosphorylation step from X∼P to ADP to form ATP at phosphorylation Sites 1 and 2" (1965, p. 169).

As the subscript number of the coupling factor suggests, Racker was also separating other factors – F_2, F_3, and F_4 – and investigating their role in phosphorylation or in other reactions such as ATP–P_i^{32} exchange. Of particular interest was factor F_o, which Racker first identified in the context of trying to account for the sensitivity of both oxidative phosphorylation and ATPase activity to oligomycin poisoning. Addition of F_o to F_1 not only provided oligomycin sensitivity, but when treated with salt solution, generated particles. I will discuss the significance of this discovery after introducing the discovery of one more morphological structure.

One More Piece of Structure and a Proposal as to Its Function

Yet another development in electron microscopy technique, the introduction of negative staining by Humberto Fernández-Morán (1962), revealed additional structure in the mitochondrion. Negative staining uses substances

[22] In his initial studies Racker collaborated with Gifford Pinchot to study oxidative phosphorylation in *Escherichia coli* in hopes of finding "a system which would withstand fractionation" (Pinchot, 1953, p. 65). They used sonic vibrations to prepare extracts and separated two components, a particulate fraction that catalyzed oxidation and a soluble fraction that was required for phosphorylation (Pinchot & Racker, 1951). Pinchot went on to study the reaction in *Alcaligenes faecalis*, where he distinguished two soluble fractions, one of which was heat labile and one of which was heat stable.

[23] This is a procedure Racker had previously used with tumor cells or bacteria. He offers an interesting characterization of his work as "instrumental research: When you run out of ideas, use a new instrument" (Racker, 1965, p. 164).

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